



Epigenetics and Lung Cancer

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ABSTRACT

Lung cancer is the leading cause of cancer-related death accounting for 30% of all cancer-related deaths. The majority of lung cancer cases (80–90%) are due to smoking. Tobacco smoke and other environmental pollutants have been recognized as risk factors for more than 80–90% of cancer incidence in men. It has been reported that DNA methylation play important role in lung carcinogenesis . Several biomarkers from various sources such as genetics, proteomics, and epigenetic approaches are in use for clinical research purposes. DNA methylation analysis is one of the valuable source for cancer biomarkers. Since current diagnostic tools are either too costly or not sensitive enough to allow for early detection. Hence there is an urgent need of major technological advances in molecular oncology for the early detection of disease because early detection of lung cancer can change the outcome of disease. This review summarizes current therapeutic knowledge in lung cancer, and recent advances in therapeutic strategies.

Keywords: lung cancer; epigenetic; epidemiology; methylation

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INTRODUCTION

Lung cancer is the leading cause of cancer-related death accounting for 30% of all cancer-related deaths¹. About 80–90% of lung cancers are due to cigarette smoking² and 20% of all lung cancers are caused by a combination of environmental as well as genetic factors. Several biomarkers from various sources such as genetics, proteomics, and epigenetic approaches are in use for clinical research purposes³. The role of epigenetic changes has been implicated in lung cancer etiology^{4,5}. Therefore it could also become useful biomarkers for the early detection and diagnosis of lung cancer and may be used as prognostic markers. Since the efficiency of current treatment of the disease is strongly depends on the time of diagnosis and the better chances of survival are possible only if a tumor has been detected at an early stage. The current diagnostic tools are either too costly or not sensitive enough to allow for early detection.^{6,7} so there is an urgent needs of major technological advances in molecular oncology for the early detection of disease since early detection of lung cancer can change the outcome of disease. Hence there is an urgent need for development of rapid and efficient method which can diagnose lung cancer at an early stage. The Novel high-throughput, quantitative assays for the detection of DNA methylation or histone tail modifications are now used to find the alterations in the lung cancer genome and identify novel cancer-related genes that may becomes an attractive targets for future treatment of lung cancer and provide new insight into the biology of lung cancers.

DNA methylation is an epigenetic event whose pattern is altered frequently in a wide variety of human cancers. DNA methylation has been reported to play an important role in lung carcinogenesis and suggesting that DNA methylation analysis will be a valuable source for cancer biomarkers.^{8,9} DNA methylation has emerged as a prime source of potential cancer-specific biomarkers and occurs in CpG rich regions called CpG islands at or near gene promoters. In addition to general hypomethylation of the genome, hypermethylation of CpG islands in gene promoter regions also occurs in cancer cells.¹⁰ Methylation is one of the most common molecular alterations in human neoplasia refers to the addition of a methyl group to the cytosine ring of those cytosines that precede a guanosine (CpG dinucleotides) to form methyl cytosine (5-methylcytosine). CpG dinucleotides are found at increased frequency in the promoter region of many genes and methylation in the promoter region is frequently associated with gene silencing.¹² Such cancer-specific hypermethylation represent differential DNA methylation profiles between tumor and non-tumor tissues which can be distinguish the tumor from non-

tumor. Hence DNA methylation serves as a cancer-specific molecular marker. Methylation of DNA is an important epigenetic process involved in fundamental biological events such as development and cell differentiation.¹³ the analysis of DNA methylation biomarkers is an emerging field that provides promising potential for improving the clinical process of lung cancer diagnosis.¹⁴ Methylation plays an important role in normal cells as well as in tumor development. In normal cells it contributes to chromatin organization, silencing of transposable elements, X chromosome inactivation, tissue-specific expression and genetic imprinting.^{15, 16} The role of epigenetic changes has been implicated in lung cancer etiology.^{17,18} Methylation has been described as an early event in lung tumorigenesis.

A well-studied example in lung cancer is the aberrant promoter methylation of the tumor suppressor gene, p16,¹⁹ H-cadherin,²⁰ death-associated protein (DAP) kinase 1 (DAPK1)²¹ the candidate tumor suppressor gene RASSF1A²², retinoic acid receptor b-2 (RARb), tissue inhibitor of metalloproteinase 3 (TIMP3), O6-methylguanine-DNA-methyltransferase (MGMT), E-cadherin (ECAD), p14ARF and glutathione S-transferase P1 (GSTP1) in primary non-small cell lung cancers.²³ It has been found that APC, CDH13, KLK10, DLEC1, RASSF1A, EFEMP1, SFRP1, RARb and p16(INK4A) genes showed significantly higher frequency of methylation in NSCLC as compared with normal tissues in Chinese population. p16 promoter methylation is proposed as a biomarker for early detection of lung cancer and monitoring of prevention^{25, 26} Using sensitive PCR-based methylation analysis, methylation in p16 and MGMT promoters was found in sputum of smokers up to 3 years prior to clinical diagnosis of squamous cell lung carcinoma²⁷

COMBINATION EPIGENETIC THERAPY:

This approach has been demonstrated the promising synergy in preclinical models. The Treatment with either the HDAC inhibitor trichostatin-A, or the hypo-methylating agent decitabine alone does not restore transcription of these genes²⁸ but the combination of these two agents reversed silencing and led to significant re-expression. In lung cancer cell lines the HDAC inhibitor depsipeptide acetylates histones H3 and H4²⁹ but this effect is significantly magnified when cells are pre-treated with decitabine. The combination of the DNMT inhibitor 5-azacytidine and the HDAC inhibitor entinostat inhibited growth of *K-ras/p53* mutant lung adenocarcinoma in an orthotopic lung cancer mouse model³⁰. Epigenetic analysis demonstrated demethylation across hundreds of genes and re-expression of several critical genes including *p16*. It is the gene reactivation properties that underlie the therapeutic efficacy of these drugs. However evidence for epigenetic activation of tumor promoting genes has been reported³¹ and

pointing to the need for a deeper understanding of epigenetic regulation and the consequences of epigenetic therapies.

MicroRNAs:

MicroRNAs are small non-coding RNAs that regulate gene expression at the post-transcriptional level^{32, 33}. A single mi-RNA can target many genes. Depending on whether it targets proto-oncogenes or tumor suppressor genes, its expression may be up or down-regulated in cancer³⁴. In lung cancer the let-7a-3 promoter was found to be hypo-methylated in lung adenocarcinoma compared to normal tissue and suggesting an oncogenic role for this mi-RNA in lung cancer³⁵. Mi-RNA can also be silenced by histone modifications. Increased level of repressive H3K27 trimethylation and H3 K9 dimethylation were seen on the miR-212 promoter in lung cancer cell line Calu-1, which expresses low levels of miR-212 compared to a human fetal lung fibroblast cell line which showing high miR-212 expression, and the effect of epigenetic modifying drugs such as HDACs supported a role for histone modifications in miR-212 regulation³⁶. While epigenetic effects can also control mi-RNA expression. DNMTs can be regulated by mi-RNAs in lung cancer cells³⁷. Based on the importance of mi-RNAs in cancer and their ability to influence gene expression they are under investigation as therapeutic tools that can potentially target any RNA of interest.

Small Cell Lung Cancer:

Most of the preclinical and clinical experience in lung cancer with epigenetic therapy has been focused on NSCLC. The combination of DNMT inhibition with HDAC inhibition was found to have a greater proapoptotic effect than monotherapy³⁸. The synergy between HDAC inhibition and various cytotoxic agents has also been demonstrated in preclinical SCLC models³⁹. In similar *in vitro* models decitabine showed a synergistic induction of DNA damage in the context of HDAC inhibition⁴⁰. The understanding of the epigenetic under pinning of SCLC development and progression increases, the development and application of epigenetic therapies are sure to follow.

CONCLUSION:

A better appreciation of the interaction between epigenetic and genetic alterations will be critical moving forward. As such studies will possible reveal additional subtypes of lung cancer that respond to unique treatment strategies, allowing refinement of treatment paradigms. Which may also provide a measure of molecular response to the drugs and will be a key element to incorporate in future studies. Further investigation of the epigenomic profiles of various

epigenetic effectors including DNA methylation, histone marks, chromatin conformation, and mi-RNAs will be of great importance for the development of new targeted therapies .

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